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Prevalence of Antibodies against Seasonal Influenza A and B Viruses in Children in Netherlands[▽]

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To gain insight into the age at which children become infected with influenza viruses for the first time, we analyzed the seroprevalence of antibodies against influenza viruses in children 0 to 7 years of age in the Netherlands. Serum samples were collected during a cross-sectional population-based study in 2006 and 2007 and were tested for the presence of antibodies against influenza A/H1N1, A/H3N2, and B viruses representative of viruses present in previous influenza seasons using the hemagglutination inhibition assay. The seroprevalence of antibodies to influenza virus was higher in children 1 to 6 months of age than in children 7 to 12 months of age, which likely reflects the presence of maternally derived antibodies. The proportion of study subjects >1 year of age with detectable antibodies against influenza viruses gradually increased with age until they reached the age of 6 years, when they all had antibodies to at least one influenza A virus. These findings may have implications for the development of vaccination strategies aiming at the protection of young children against seasonal and/or pandemic influenza virus infection.

Infection with influenza viruses is an important cause of illness in children, with estimated annual attack rates in this age group ranging from 20 to 30% during epidemics (9, 11). Young children with underlying disease are especially at risk for severe disease after infection with an influenza virus, but it has also been demonstrated that the hospitalization rates attributable to influenza virus infection observed among young children without underlying disease are similar to those observed among older adults (18, 23). Furthermore, the importance of influenza as a cause of severe disease was demonstrated during the 2003-2004 influenza season, when a newly emerged drift variant caused an unusually high number of severe fatal cases of influenza among children (19). In addition, the pandemic caused by the influenza A/H1N1(2009) virus has highlighted the importance of influenza viruses as a cause of morbidity and mortality in infants (2, 12).

Furthermore, since children have a high number of contacts relative to other age groups and have a tendency to make contacts within their own age group, they may have the highest incidence of infection after the introduction of a newly emerging virus (22). In addition, they may also shed virus for a prolonged period of time and have higher virus loads in the nasopharynx (10, 14). Therefore, children most probably play an important role in the transmission of virus and are considered efficient vectors for spreading the disease.

To prevent morbidity and mortality of children due to infection with influenza viruses, a number of countries, including the United States, have recommended vaccinating all healthy children 6 to 59 months of age against influenza (8, 15). In various studies, it has been demonstrated that annual vaccination against seasonal influenza is beneficial for children and reduces the transmission of virus (21, 27, 33, 35, 37, 43). However, the impact of vaccination will be influenced by the immune status of the vaccinated individuals. Since they will be more at risk to become infected and develop disease, naïve subjects most likely will benefit from vaccination more than children who have already experienced an infection with one or more influenza viruses. In addition, it can be anticipated that with increasing age the chance of having experienced an influenza virus infection also increases. However, at present it is not fully clear at which age children become infected for the first time and develop influenza virus-specific immunity, and detailed seroepidemiological studies of this age group are largely lacking (36, 42). Here we report on the seroprevalence of antibodies against influenza A/H1N1, A/H3N2, and B viruses in children from 1 month to 7 years of age in the Netherlands. To this end, serum samples that were collected during a cross-sectional population-based study designed to represent the population of the Netherlands were used (40). These serum samples were tested for the presence of antibodies against representative influenza A/H1N1, A/H3N2, and B viruses from multiple influenza seasons using the hemagglutination inhibition (HI) assay, which is the "gold standard" for the demonstration of antibodies against influenza viruses (3). In addition, we were able to discriminate between antibodies against various antigenically distinct influenza A/H1N1 and influenza A/H3N2 viruses and antibodies to influenza B viruses from the B/Victoria/2/87 and B/Yamagata/16/88 lineages (referred to here as the Victoria and Yamagata lineages, respectively). In children >1 year of age, there was a gradual, age-related in-

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crease in the seroprevalence of antibodies against all influenza viruses until antibodies against at least one influenza virus were detected in all children >6 years of age. Results obtained in this study give more insight into the rate of infection of children with influenza viruses during nonpandemic seasons and may aid policy making regarding the implementation of vaccination strategies in this vulnerable age group.

MATERIALS AND METHODS

Collection of serum samples. Serum samples were collected during a nationwide cross-sectional population-based study which was performed in the Netherlands from February 2006 to June 2007 (PIENTER 2 Study) to evaluate the Dutch national immunization program (40). For this purpose, serum samples were collected from, in total, 6,386 individuals (aged 0 to 79 years, men and women). For our study, 720 serum samples obtained from children 0 to 7 years of age were used. Fifty-six samples were obtained from children 1 to 6 months of age, and 98 serum samples were obtained from children 7 to 12 months of age. The numbers of samples obtained from children who were 1, 2, 3, 4, 5, 6, and 7 years of age were 57, 80, 93, 91, 72, 75, and 97, respectively.

Selection of representative influenza viruses. Representative influenza A/H3, A/H1, and B viruses that circulated in the Netherlands in seasons 1999-2000 to 2006-2007 were selected on the basis of data collected by the National Influenza Center for the World Health Organization (WHO) in the Netherlands (4–7, 29–32). For most seasons, vaccine strains were used, but when epidemiological strains that gave higher antibody titers with reference ferret serum could be identified, these were included as well (Table 1). Furthermore, influenza B viruses of both the Victoria and Yamagata lineages were used for each year, although in some seasons only influenza B viruses belonging to one lineage were detected in clinical specimens in the Netherlands. In addition, data collected by the Dutch national surveillance program were used to assess the severity of the influenza seasons and to evaluate the relative dominance of each of the influenza virus types and subtypes.

Before use in the HI assay, vaccine strains were inoculated in the allantoic cavity of 11-day-old embryonated chicken eggs, while epidemiological strains were propagated in confluent Madin-Darby canine kidney (MDCK) cells. Allantoic fluid was harvested after 2 days, and culture supernatant was harvested after cytopathologic changes were complete. Both the allantoic fluid and culture supernatant were cleared by low-speed centrifugation. Sera from children between 1 month and 12 months of age were tested for the presence of antibodies against all influenza viruses representative of those present in the six preceding influenza seasons to analyze them for the presence of maternal antibodies, while serum samples collected from children older than 1 year were tested for the presence of antibodies against all influenza viruses present in seasons in which they might have been exposed according to their age (Table 1).

Serological testing. Serum samples were tested for the presence of antibodies against the hemagglutinin of the respective influenza viruses by HI assay as described previously (25). In brief, serum samples were treated with cholera filtrate and heat inactivated at 56°C for 1 h. Duplicate 2-fold serial dilutions of pretreated serum samples were subsequently incubated with 4 hemagglutination units of an influenza virus or phosphate-buffered saline (PBS) for 30 min at 37°C, and subsequently, 1% turkey erythrocytes were added. Hemagglutination patterns were read after incubation for 1 h at 4°C. The highest dilution of serum that still gave complete inhibition of the hemagglutination was recorded as the titer, and when duplicate results were different, geometric mean titers (GMTs) were calculated.

Serum samples were considered negative when they failed completely to inhibit agglutination of erythrocytes (antibody titer < 10) by any of the selected viruses. Serum samples collected from ferrets before and after infection with each of the influenza viruses were used as negative and positive controls, respectively, in the HI assay.

Statistical analysis. Pearson's correlation coefficient was used to calculate correlations between the titers of antibodies detected against multiple variants of influenza A/H3N2, A/H1N1, and B viruses. Furthermore, assuming a binominal distribution, the two-sided exact 95% confidence interval (CI) was calculated for the seroprevalences of antibodies against influenza A/H3N2, A/H1N1, and B viruses using Stata/SE software, version 11.0. Statistical analysis of differences between children 1 to 6 months of age and children 6 to 12 months of age was performed using the chi-square test. The Cochrane-Armitage trend test was performed using SAS software, version 9.2, to evaluate whether an age-related trend in the presence of antibodies against influenza viruses was present.

TABLE 1. Influenza virus epidemics in the Netherlands during influenza seasons from 1999 to 2007

Season	Selected influenza virus			Severity	Dominant (sub)type[a]			Dominant B lineage	Age (yr) at which a child may be exposed
	A/H3N2	A/H1N1	B		A/H3N2	A/H1N1	B		
2006-2007	A/Hiroshima/52/05	A/NewCaledonia/20/99, A/Solomon Islands/3/2006	B/Malaysia/2506/04, B/Neth/001/07, B/Jiangsu/10/03	Moderate	D	LA	LA	Yamagata only	0–<8
2005-2006	A/New York/55/04	A/NewCaledonia/20/99, A/Neth/239/06	B/Malaysia/2506/04, B/Jiangsu/10/03	Moderate	CD	LA	CD	Yamagata+ Victoria	0–<8
2004-2005	A/Wyoming/3/03	A/NewCaledonia/20/99	B/Malaysia/2506/04, B/Jiangsu/10/03	Relative severe	D	LA	LA	Yamagata only	1–<8
2003-2004	A/Wyoming/3/03	A/NewCaledonia/20/99, A/Neth/128/04	B/Malaysia/2506/04, B/Jiangsu/10/03	Moderate	D	NI	LA	Yamagata only	2–<8
2002-2003	A/Panama/07/99	A/NewCaledonia/20/99	B/Shangdong/7/97	Moderate	CD	LA	CD	Victoria only	3–<8
2001-2002	A/Panama/07/99	A/NewCaledonia/20/99	B/Guangdong/120/00, B/Neth/080/02	Moderate	D	LA	LA	Yamagata+ Victoria	4–<8
2000-2001	A/Panama/07/99, A/Neth/118/01	A/NewCaledonia/20/99, A/Neth/306/00	B/Yamanashi/166/98, B/Neth/429/99	Mild	LA	D	LA	Yamagata only	5–<8
1999-2000	rA/Sydney/5/97	A/Beijing/262/95, A/Johannesburg/82/96	B/Yamanashi/166/98, B/Neth/429/99	Moderate	D	LA	LA	Yamagata only	6–<8

[a] D, dominant; CD, codominant; LA, low activity; NI, no viruses of this subtype isolated.

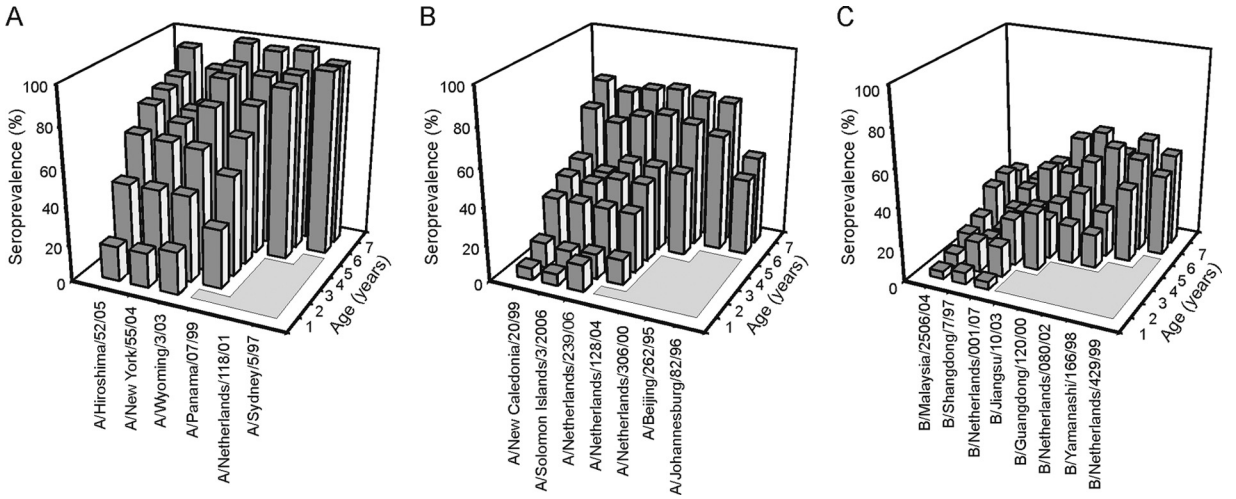

FIG. 1. Seroprevalence of antibodies against individual influenza viruses. Serum samples from children 0 to 7 years of age were tested for the presence of antibodies against representative influenza A/H3N2 (A), A/H1N1 (B), and B (C) virus strains. For each age group, representative influenza viruses to which they may have been exposed were selected according to their age. Indicated are the strains that have been used to evaluate serum samples for the presence of antibodies and the percentage of serum samples in which antibodies against each influenza virus antigen were detected. Shaded areas, not tested.

RESULTS

Influenza epidemics from 1999 to 2007 in the Netherlands. Using epidemiological and virological data, we were able to assess the relative severity of the influenza epidemics in the Netherlands from 1999 to 2007 and the causative viruses. During most seasons, influenza viruses caused moderate epidemics, except for the 2004-2005 season, which was relatively severe and predominantly caused by influenza A/H3N2 viruses, and the 2000-2001 season, which was relatively mild and caused by influenza A/H1N1 viruses. Furthermore, most seasons were dominated by influenza A/H3N2 viruses, while during the 2002-2003 and 2005-2006 influenza seasons, both influenza A/H3N2 and B viruses were codominant. During most seasons, the majority of isolated influenza B viruses belonged to the Yamagata lineage. However, during the 2002-2003 influenza season, in which influenza B viruses were codominant, only viruses from the Victoria lineage were isolated in the Netherlands. During most epidemics from 1999 to 2007, influenza A/H1N1 viruses caused only low influenza activity, except during the 2000-2001 season (Table 1).

Age-dependent seroprevalence of antibodies against individual influenza virus strains. First, the prevalence of antibodies directed against individual influenza virus strains was assessed using serum samples from children 1 to 7 years of age. Strains against which the study subjects potentially could have developed an antibody response on the basis of their age at the time point of sampling were used. As shown in Fig. 1A, an age-dependent increase in the proportion of subjects with antibodies to selected A/H3N2 strains was observed. The highest prevalence of antibodies to a single strain was observed against influenza viruses A/NL/118/01 and A/Wyoming/3/03 (100%) in subjects 7 years old.

A similar pattern was observed for the prevalence of antibodies to individual influenza A viruses of the H1N1 subtype, although the overall seroprevalence was lower (Fig. 1B). The highest seroprevalence of antibodies to individual strains was observed for influenza viruses A/NL/128/04 (77%) in subjects 7 years of age, and this seroprevalence was similar to that of antibodies against most other H1N1 strains.

The seroprevalence of antibodies to individual influenza B virus strains displayed a different pattern and was largely dependent on the lineage of the influenza B virus that was used. In general, higher seroprevalences of antibodies against influenza B viruses of the Yamagata lineage than against viruses of the B/Victoria lineage (B/Malaysia/2506/04 and B/Shangdong/7/97) were detected (Fig. 1C).

Seroprevalence during first year of life. Serum samples from children 1 to 12 months of age were tested for the presence not only of antibodies to influenza viruses from the 2006-2007 season but also of those specific to older strains, since it was anticipated that these sera also might contain maternally derived antibodies.

In 15% (CI = 6 to 27%) of the children between 1 and 6 months of age, antibodies against at least one of the influenza A/H1N1 viruses tested were detected, while only 4% (CI = 1 to 10%) of children between 7 and 12 months of age had antibodies against A/H1N1 viruses (Fig. 2A and C). In 43% (CI = 30 to 57%) and 36% (CI = 23 to 50%) of the children 1 to 6 months of age, antibodies against at least one influenza A/H3N2 virus and one B virus, respectively, were detected. For the serum samples obtained from children 7 to 12 months of age, the proportions of subjects with antibodies to these viruses were 19% (CI = 12 to 28%) and 5% (CI = 2 to 12%), respectively (Fig. 2A). The significant differences in the prevalence of antibodies to A/H3N2 and B viruses between the two age groups could be largely attributed to a difference in the proportion of serum samples containing antibodies to strains from previous influenza seasons, like A/Wyoming/3/03, A/Panama/07/99, A/Sydney/5/97 (all A/H3N2), and B/Yamanashi/429/99 (Fig. 2B and D). This indicates that the relatively high seroprevalence of antibodies in children 1 to 6 months of age can indeed be attributed to maternally derived antibodies.

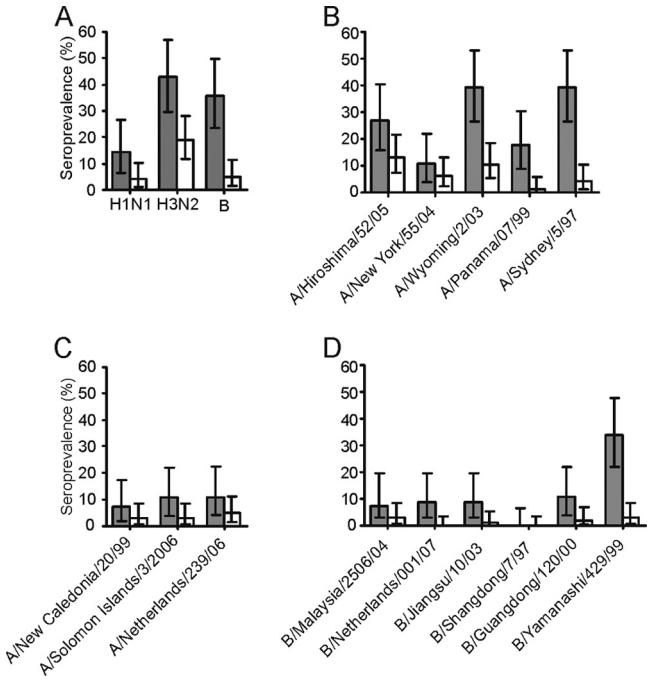

FIG. 2. Seroprevalence of antibodies against influenza viruses in children 1 to 12 months of age. (A) Seroprevalences of antibodies against influenza A/H3N2, A/H1N1, and B viruses of the 2000 to 2007 influenza seasons in children 1 to 6 months of age (gray bars) and 7 to 12 months of age (white bars). Serum samples were tested for the presence of antibodies against multiple antigens, as is indicated for influenza A/H3N2 (B), influenza A/H1N1 (C), and B (D) viruses. Bars indicate the percentage of the serum samples in which antibodies were detected, and error bars indicate the 95% confidence intervals.

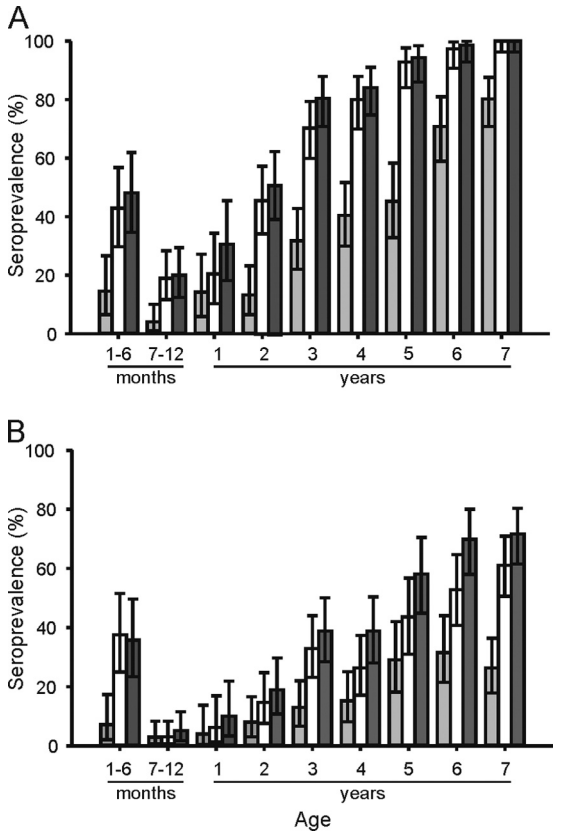

FIG. 3. Seroprevalence of antibodies against influenza A and B viruses depends on age. (A) The percentages of serum samples from children in which antibodies against at least one of the representative influenza viruses were detected were calculated for influenza A/H1N1 viruses (light gray bars), influenza A/H3N2 viruses (white bars), and all influenza A viruses (dark gray bars). (B) The same procedures were used to calculate the seroprevalence of antibodies against at least one of the influenza B viruses from the Victoria lineage (light gray bars) and the Yamagata-lineage (white bars) and all influenza B viruses (dark gray bars). Bars indicate the percentage of the serum samples in which antibodies were detected, and error bars indicate the 95% confidence intervals.

Age-dependent seroprevalence of antibodies to any influenza A or B virus. The seroprevalence of antibodies to individual influenza virus strains was used to calculate the proportion of subjects with antibodies to at least one influenza A or B virus. Within the influenza A viruses, the relative contributions of antibodies to influenza A/H3N2 and A/H1N1 viruses were discriminated, and within the influenza B viruses, those to the Yamagata and Victoria lineages were discriminated.

As shown in Fig. 3, the seroprevalence of antibodies to influenza A viruses declined after 6 months of age. Thereafter, with increasing age the proportion of subjects with antibodies to influenza A viruses increased steadily. At the age of 6 years, virtually all subjects (99%; CI = 93 to 100%) had developed antibodies to an influenza A virus. For subjects >2 years of age, the proportion with antibodies to influenza A/H3N2 viruses was significantly higher than the proportion with antibodies to A/H1N1 viruses.

A similar pattern was observed for the development of antibodies to influenza B viruses. After 6 months, the proportion of subjects with antibodies to an influenza B virus dropped to 5% (CI = 2 to 12%). With increasing age, a gradual incline in the proportion of children with antibodies to influenza B virus was observed. At age 7 years, 72% (CI = 61 to 80%) of the subjects had developed antibodies to at least one influenza B virus. The seroprevalence of antibodies to influenza B viruses of the Yamagata lineage was higher than that of antibodies to the Victoria lineage. Using the Cochrane-Armitage trend test, the presence of a significant age-related trend in the increase of the seroprevalence of antibodies to influenza A/H1N1, A/H3N2, and B viruses was demonstrated ($P < 0.01$).

Estimated attack rates. The differences in seroprevalence of antibodies to the respective influenza viruses at various ages were used to estimate attack rates. The proportion of children with antibodies to influenza A/H3N2 viruses increased only 1.5% between children 7 to 12 months of age and children 1 year of age. The highest increase in the seroprevalence of antibodies against influenza A/H3N2 viruses was observed at ages 2 and 3 years. At these ages, the proportion of subjects with antibodies increased by 25% each year. The highest increases in the seroprevalence of antibodies to influenza A/H1N1 viruses were observed at age 3 years (18%) and at age 6 years (26%). During the first year of life, only a minority of the subjects acquired antibodies to influenza B viruses (5%). The highest increase in the seroprevalence of antibodies against influenza B viruses was observed at ages 3 years (20%) and 5 years (19%). These increases could be largely attributed

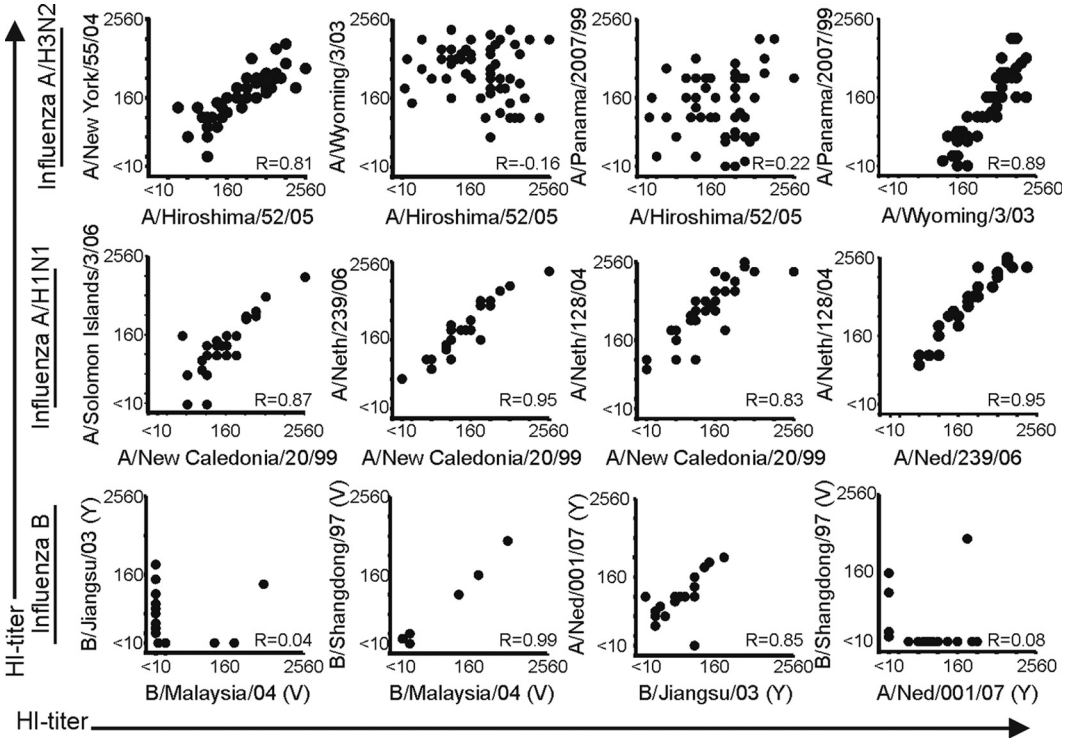

FIG. 4. Correlation of titers of antibodies against individual influenza A virus strains in 4-year-old children. Correlations between the titers of antibodies against multiple representative influenza A/H3N2 viruses, influenza A/H1N1 viruses, and influenza B viruses are shown. Dots indicate individual serum samples, and the Pearson correlation coefficient was calculated for all data points for which antibodies against at least one influenza virus was detected. For influenza B viruses, the letter behind the name of each strain indicates the lineage to which the virus belongs (V, Victoria lineage; Y, Yamagata lineage). Neth and Ned, Netherlands.

to the development of antibodies directed against influenza B viruses of the Yamagata lineage. In general, the increase in the seroprevalence of antibodies against viruses from the Victoria lineage was modest, with the exception of a 14% increase observed in subjects 5 years of age.

Correlation between antibody titers against multiple influenza virus strains. As serum samples were tested for antibodies against various influenza viruses, we determined the correlation between antibody titers against different strains within a (sub)type (Fig. 4). In general, the titers of antibodies against various influenza A/H1N1 viruses correlated well ($R > 0.8$), as did those of antibodies against strains of each of the lineages of influenza B virus ($R > 0.8$). In contrast, the titers of antibodies against viruses from the two different influenza B virus lineages correlated poorly ($R < 0.1$), although in some samples antibodies against viruses from both lineages were detected. The correlation of antibody titers against different influenza A/H3N2 viruses was dependent on the year of isolation and most likely on the antigenic match between the two strains that were studied. For example, good correlations between titers against A/New York/55/04 and A/Hiroshima/52/05 and between A/Panama/2007/00 and A/Wyoming/3/03 were observed, whereas titers between A/Wyoming/3/03 or A/Panama/2007/99 and A/Hiroshima/52/05 correlated poorly. Figure 4 shows an example of the correlations between antibody titers that were observed with the serum samples obtained from children 4 years of age.

DISCUSSION

In the present study, the seroprevalence of antibodies against influenza viruses was investigated in children in the Netherlands. Sera were collected from February 2006 to June 2007 in a cross-sectional population-based study and were tested for the presence of antibodies against influenza virus strains representative of viruses that circulated during the life spans of the children tested. Since the persistence of maternally derived antibodies is short-lived and is probably less than 6 months (17), sera from children <12 months of age were also tested for antibodies against older influenza viruses that may have infected their mothers.

Indeed, the seroprevalence of antibodies to influenza viruses was relatively high in children between 1 and 6 months of age. This could be attributed to the presence of maternally derived antibodies to older influenza virus strains. The seroprevalence was lower in children between 6 months and 1 year of age but showed an age-dependent increase until the age of 7 years, when all of the children had developed antibodies to at least one influenza A virus and 72% had developed antibodies to at least one influenza B virus. The increase in the seroprevalence was not caused by differences in the GMTs of antibodies against influenza viruses, since the GMTs of antibodies against the respective strains were independent of age. High antibody titers were also observed in serum samples collected from

some children at from 7 to 12 months of age, reflecting recent infections with the corresponding viruses.

In children of all ages, the seroprevalence of antibodies against influenza A/H3N2 viruses was higher than the seroprevalence of antibodies against influenza A/H1N1 or B viruses. This is in accordance with epidemiological data from the Netherlands collected between 1999 and 2007. During influenza seasons in this period, antibodies to influenza A/H3N2 viruses were predominantly detected in clinical specimens when their seroprevalence was compared to that of antibodies to influenza A/H1N1 and influenza B viruses. In addition, we observed a relatively strong increase in the seroprevalence of antibodies against influenza A/H1N1 viruses in children 6 years of age compared to that in children of other ages, which could be attributed to the dominant circulation of influenza A viruses of this subtype during the 2000-2001 influenza season. Furthermore, the presence of antibodies to influenza B viruses of the B/Yamagata lineage and the B/Victoria lineage could be discriminated. These two lineages are antigenically distinct and cross-react poorly (20, 34). In addition, in young children who had most likely been infected with only one influenza B virus, only antibodies against influenza B viruses of a single lineage were detected. In older children, antibodies against influenza B viruses of both lineages were detected, which is in accordance with the possibility that these children had subsequently been infected with both viruses during their lives. Overall, the seroprevalence of antibodies to influenza B viruses of the B/Yamagata lineage is higher than the seroprevalence of those specific for viruses of the B/Victoria lineage. This correlates with epidemiological data, which indicate that in five out of eight seasons under investigation, only viruses from the B/Yamagata lineage were isolated and in two other seasons viruses of both lineages were codominant.

Assuming that children who were infected with influenza viruses also developed antibodies against the corresponding viruses, we calculated the estimated attack rates on the basis of the seroconversion rates at the respective ages. Influenza A/H3N2 viruses had the highest attack rates in children who were between 2 and 4 years old. However, the possibility that the attack rate of older children was underestimated cannot be excluded, since subsequent infection with viruses of the same subtype may have remained undetected due to the presence of antibodies induced by previous infections. The estimated attack rates based on the seroconversion rates are comparable to the attack rates during interpandemic influenza seasons reported by others (9, 11). Strikingly, in children <2 years of age, the attack rates were relatively low compared to those in older children. Since the length and severity of the influenza seasons between 2004 and 2006 were not different from those of most other seasons and antibody titers in seropositive subjects were not age dependent, differences in exposure to influenza viruses may explain the observed differences in attack rates. To account for potential confounding, differences in the length and severity of flu seasons experienced between each age group, determined by use of information on birth and sample collection dates, were used to calculate the duration of the flu season that each subject would have experienced. Further, these values were weighted using influenza-like illness data (24) as a measure of epidemic severity during each weekly period. When these values were used to control for differences in circulating flu conditions throughout the lives of the subjects forming each year group, similar patterns of increases in seroprevalence were still encountered (Fig. 5). Of note, when a threshold of an HI titer of ≥40 instead of an HI titer of ≥10 was used for seropositivity, essentially the same results were obtained, since infection-induced antibody titers were generally higher than 40.

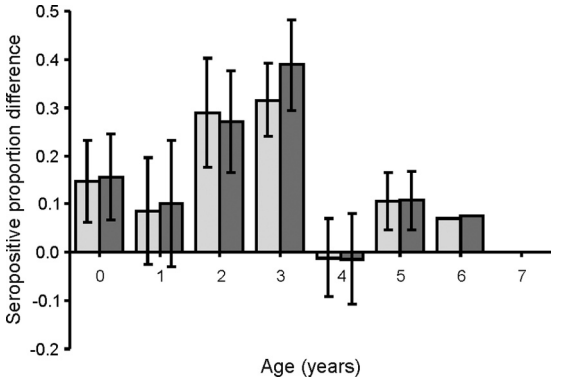

FIG. 5. Difference in proportion of seropositive individuals for each age group compared to proportion for the previous age group. Unadjusted (light gray) and adjusted (dark gray) proportions controlled for estimated differences in the severity of flu incidence throughout the lives of individuals in each group. For the adjustment, the mean total weighted influenza season time experienced by the individuals of each age group was first calculated using information about the date of birth and date of sample collection and relevant influenza-like illness data. Next, the differences in this mean for each age group compared to the mean for the previous age group were calculated, alongside an overall mean difference between age groups. Finally, the adjustments were made by scaling the value for each age group by the factor by which it differed from the overall mean for the data set, to account for age groups that had lived through a time of abnormally high or low flu incidence. For age 0, only individuals more than 220 days old were included to reduce the chance of detecting potential maternal immunity rather than genuine exposure, and values for age 0 were plotted assuming a previous seroprevalence of 0%. Error bars indicate the 95% confidence intervals.

In addition, vaccination against seasonal influenza is currently recommended in the Netherlands only for children who are at high risk for developing complications after infection with influenza virus due to underlying disease and is therefore considered a minor confounding factor in the present study.

Our results regarding the relatively high seroprevalence of antibodies in infants <7 months of age coincide with those reported for newborns (13, 41), and it is likely that transplacentally acquired maternal antibodies can protect young infants to a certain extent (26, 28). The high seroprevalence of antibodies in children <7 months of age is explained by the presence of antibodies to older influenza viruses to which their mothers may have been exposed. In addition, since vaccination against influenza is not recommended for (pregnant) mothers in the Netherlands, the proportion of vaccinated mothers is most likely very low. Indeed, the titers of antibodies to these older strains decline rapidly and are not detectable in children 7 to 12 months of age. The presence and duration of maternal antibodies against influenza have been demonstrated previously (38, 39, 41). It is unlikely that the children <7 months of

age had experienced an infection with influenza viruses, since the day of birth and day of sample collection were in between two influenza seasons for 20 of these children, including 14 with antibodies to various older influenza virus strains. In addition, two children >7 months of age were seronegative and may not have been exposed to influenza viruses for the same reason. The presence of maternal antibodies against various influenza A and B viruses in infants <7 months of age seems to be a paradox compared with the high hospitalization rate for this age group (23). However, in a substantial proportion of these infants (30%), antibodies against any influenza virus were not detectable, and these may constitute subjects highly susceptible to infection with influenza virus.

As expected, the titers of antibodies against antigenically related influenza A and B viruses correlated well. In contrast, antibodies to influenza B viruses of the B/Yamagata and B/Victoria lineages did not cross-react. Furthermore, a poor correlation was observed when the titers of antibodies against antigenically distinct A/H3N2 viruses were compared. Apparently, there is heterogeneity in the antibody repertoire of various subjects, which dictates the level of cross-reactivity with different influenza viruses.

Collectively, in this study we determined the seroprevalence of antibodies against various influenza viruses in children from 0 to 7 years of age during nonpandemic influenza seasons. We demonstrated that at 7 years of age, all children developed antibodies against at least one of the influenza viruses for which they were tested. Furthermore, the highest attack rates, calculated on the basis of the seroprevalence of antibodies to influenza A viruses, were observed in children 2 and 3 years of age. These data provide information on the age at which children experience their first infections with influenza viruses and develop immunity to these viruses. This type of information may aid decision making for the implementation of vaccination strategies that aim at achieving optimal protective immunity against seasonal and pandemic influenza. Ideally, in infants, vaccines that induce not only antibodies to seasonal influenza viruses but also immunity to influenza A viruses of other subtypes will be used (1, 16).

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